08 347780

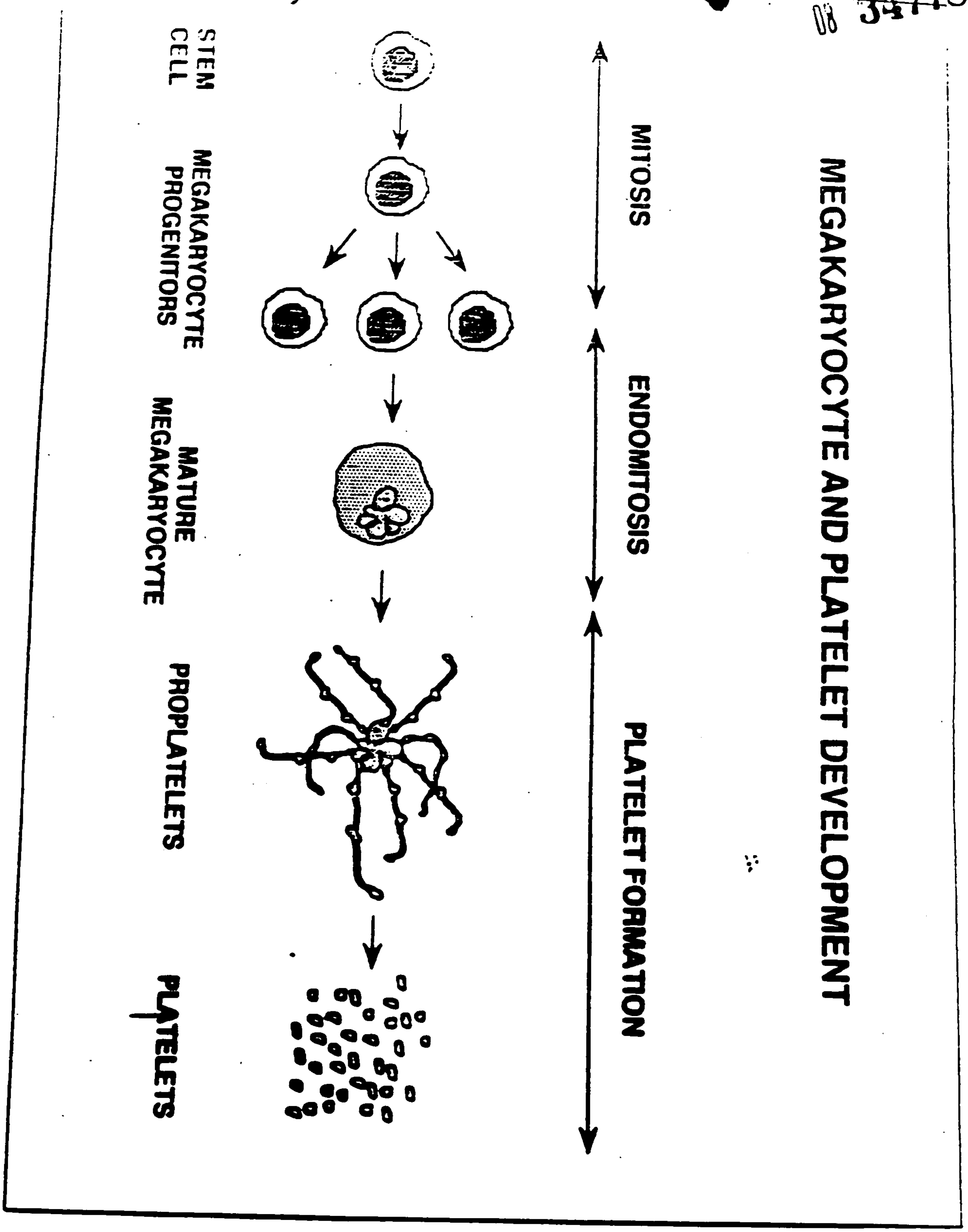


Figure 1

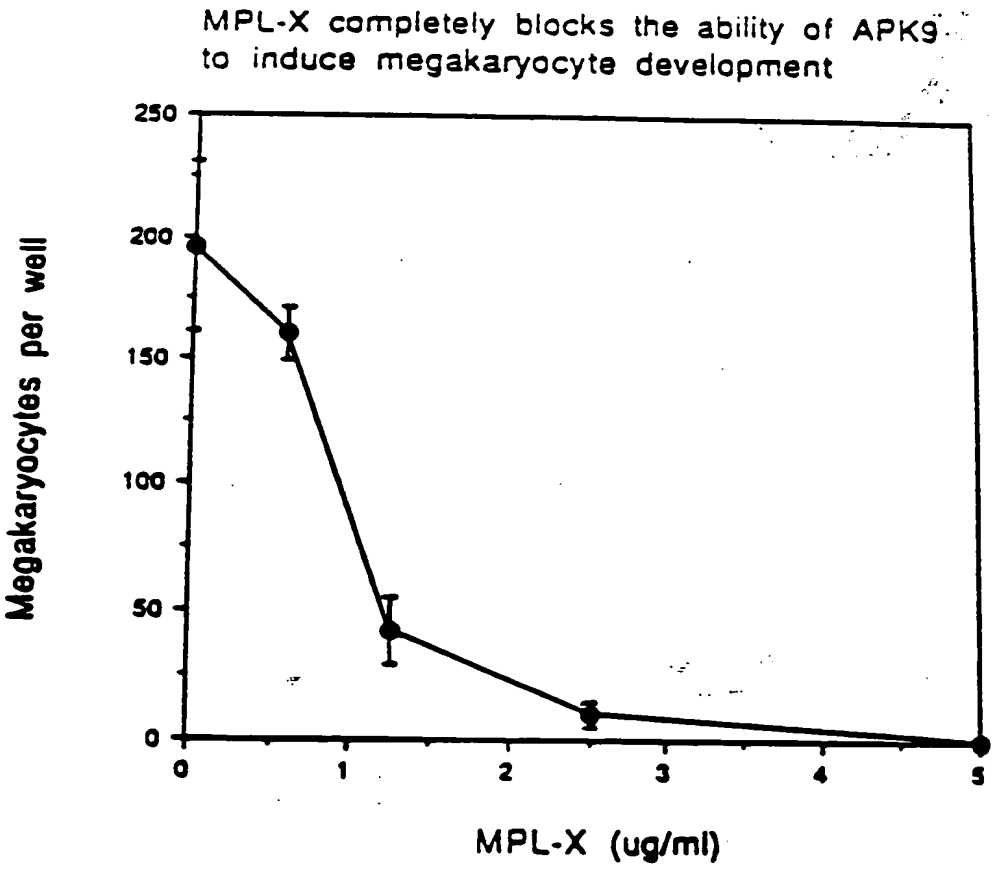

MPL-X completely blocks the ability of APK9 to induce megakaryocyte development
Megakaryocytes per well
250
200
150
100
50
0
0
1
2
3
4
5
MPL-X (ug/ml)

Figure 2

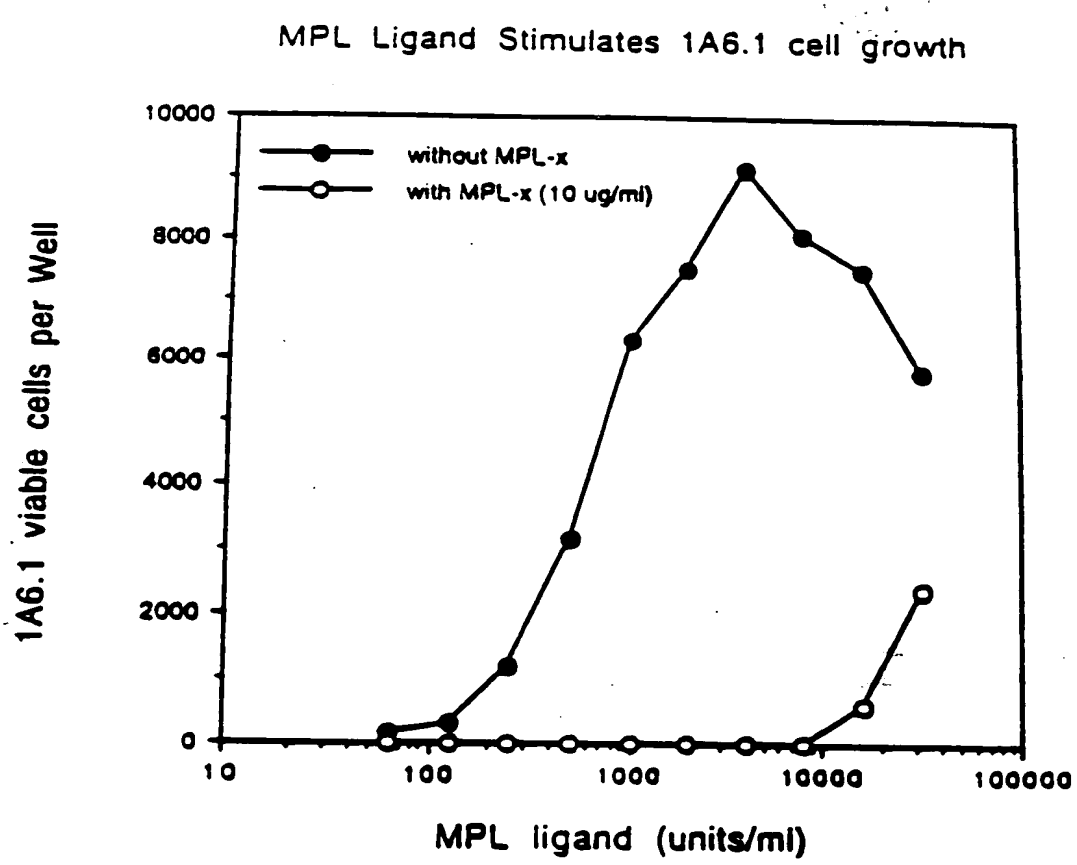

MPL Ligand Stimulates 1A6.1 cell growth
1A6.1 viable cells per Well
10000
8000
6000
4000
2000
0
without MPL-x
with MPL-x (10 ug/ml)
10
100
1000
10000
100000
MPL ligand (units/ml)

Figure 3

Purification of Mpl ligand

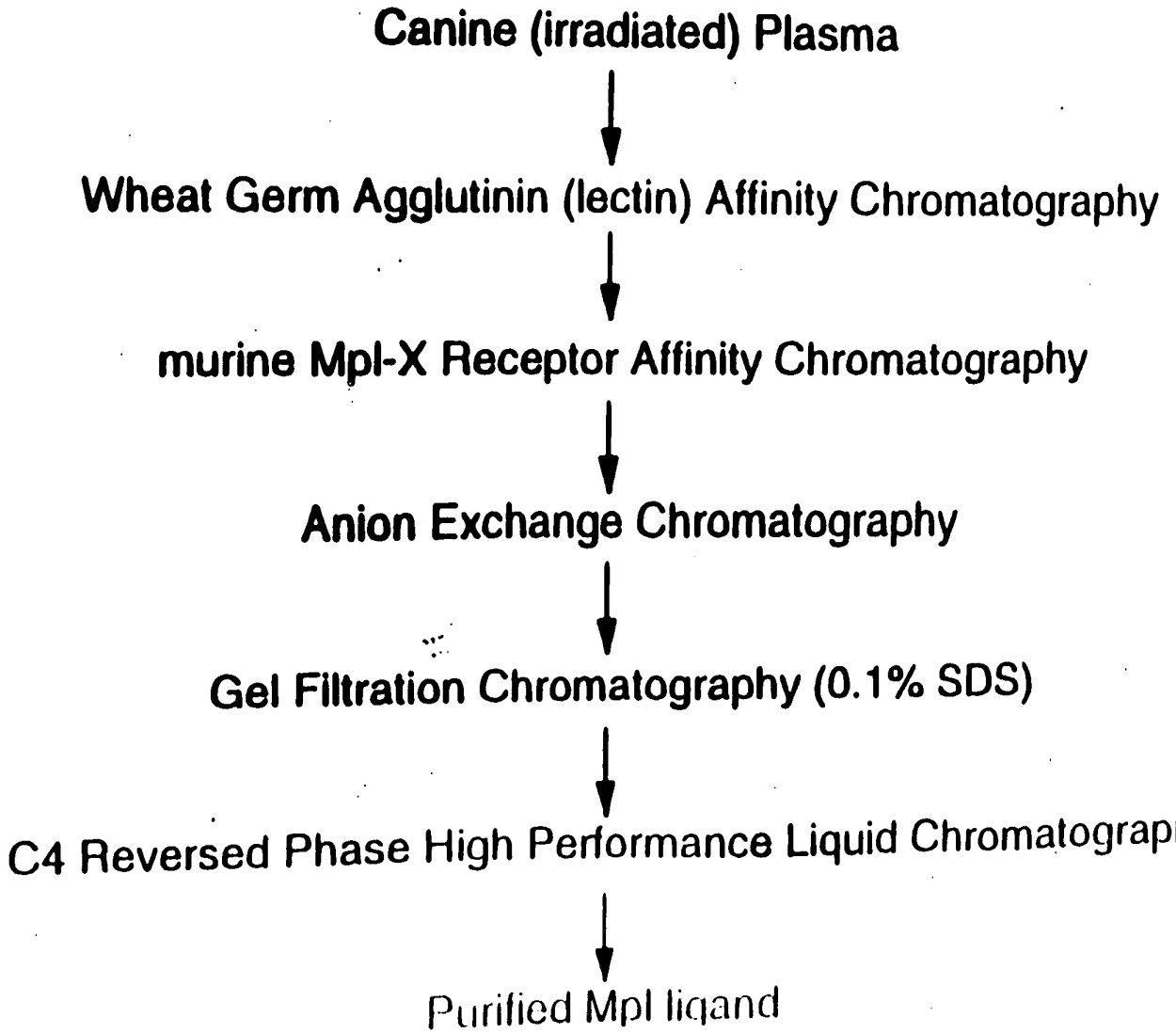
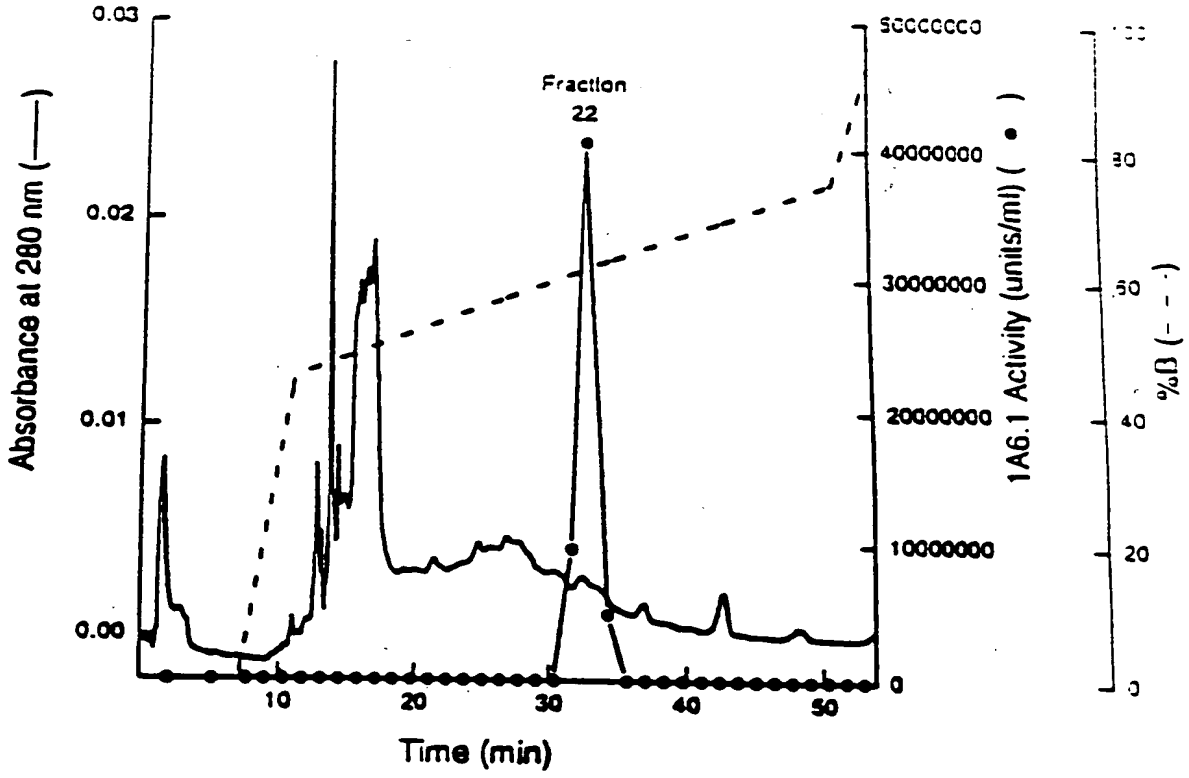
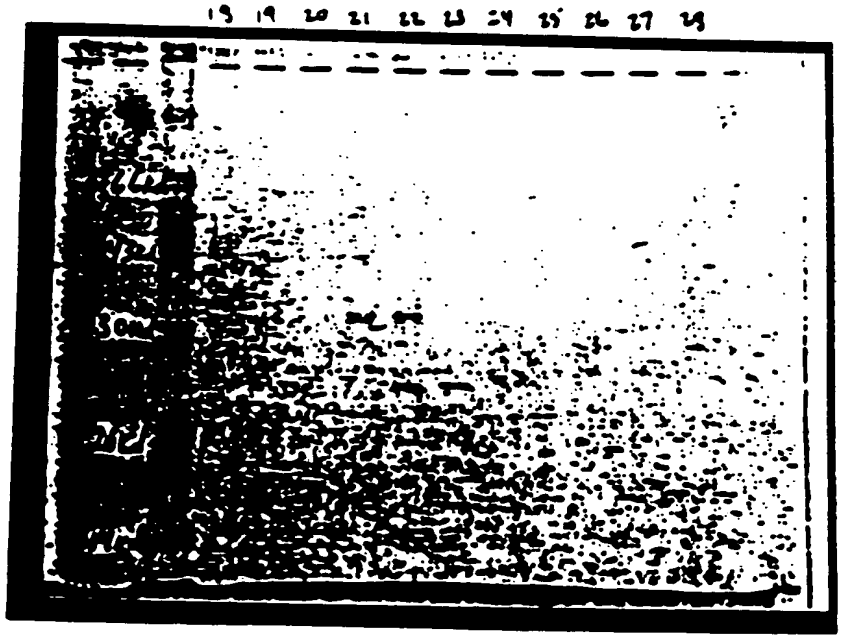


Figure 4

C4 RP-HPLC Purification of Mcl Ligand
Sample = Superdex 200 Fraction 42





SDS-PAGE 14% NON-REDUCING

Figure 5

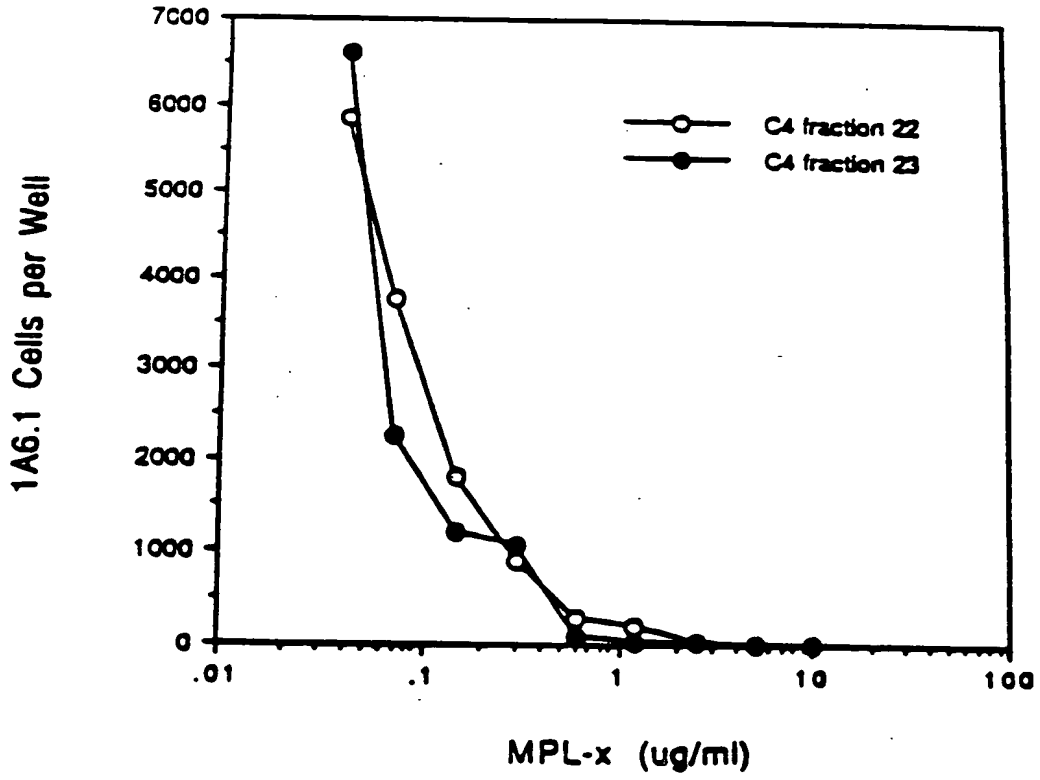

7000
6000
5000
4000
3000
2000
1000
0
1A6.1 Cells per Well
.01
.1
1
10
100
MPL-x (ug/ml)
C4 fraction 22
C4 fraction 23

Figure 6

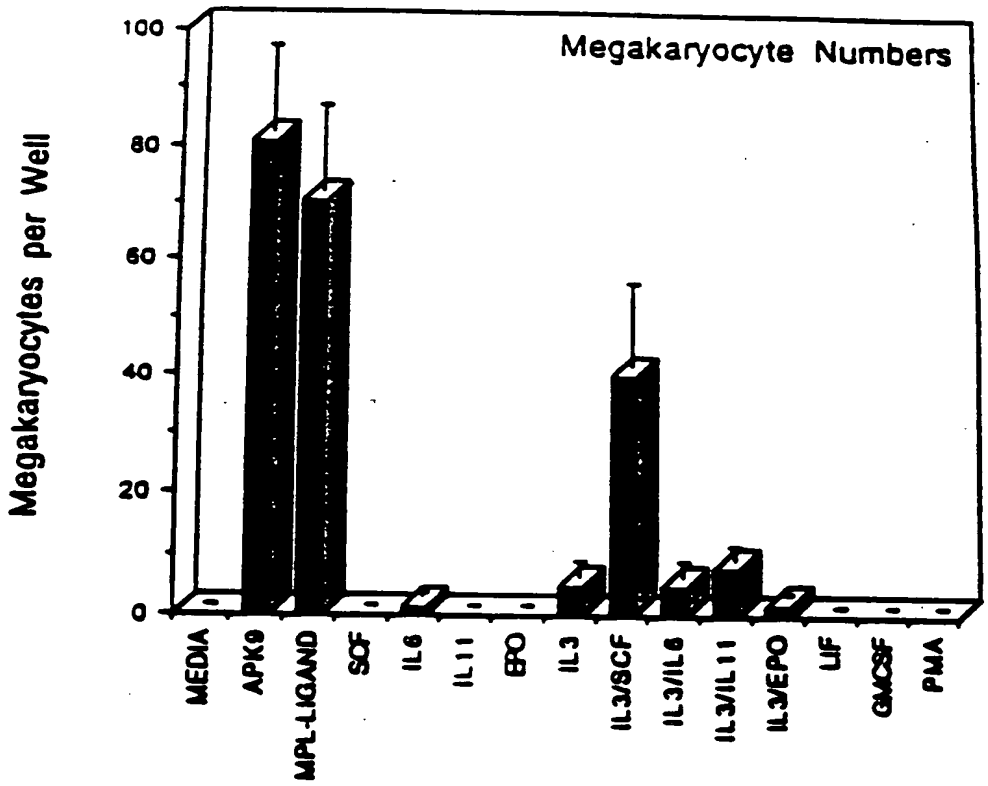

Megakaryocyte Numbers
Megakaryocytes per Well
100
80
60
40
20
0
MEDIA
APK9
MPL-LIGAND
SCF
IL6
IL11
EPO
IL3
IL3/SCF
IL3/IL6
IL3/IL11
IL3/EPO
LIF
GMCSF
PMA

Figure 7

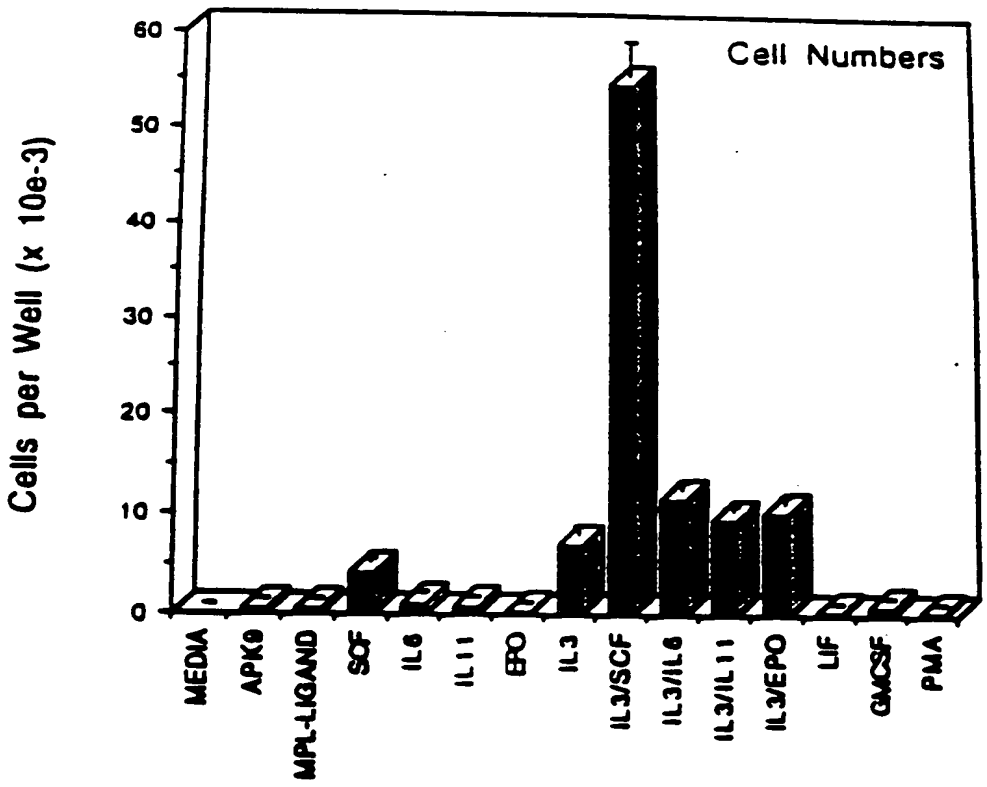

Cell Numbers
Cells per Well (x 10e-3)
60
50
40
30
20
10
0
MEDIA
APK9
MPL-LIGAND
SCF
IL6
IL11
EPO
IL3
IL3/SCF
IL3/IL6
IL3/IL11
IL3/EPO
LIF
GMCSF
PMA

Figure 8

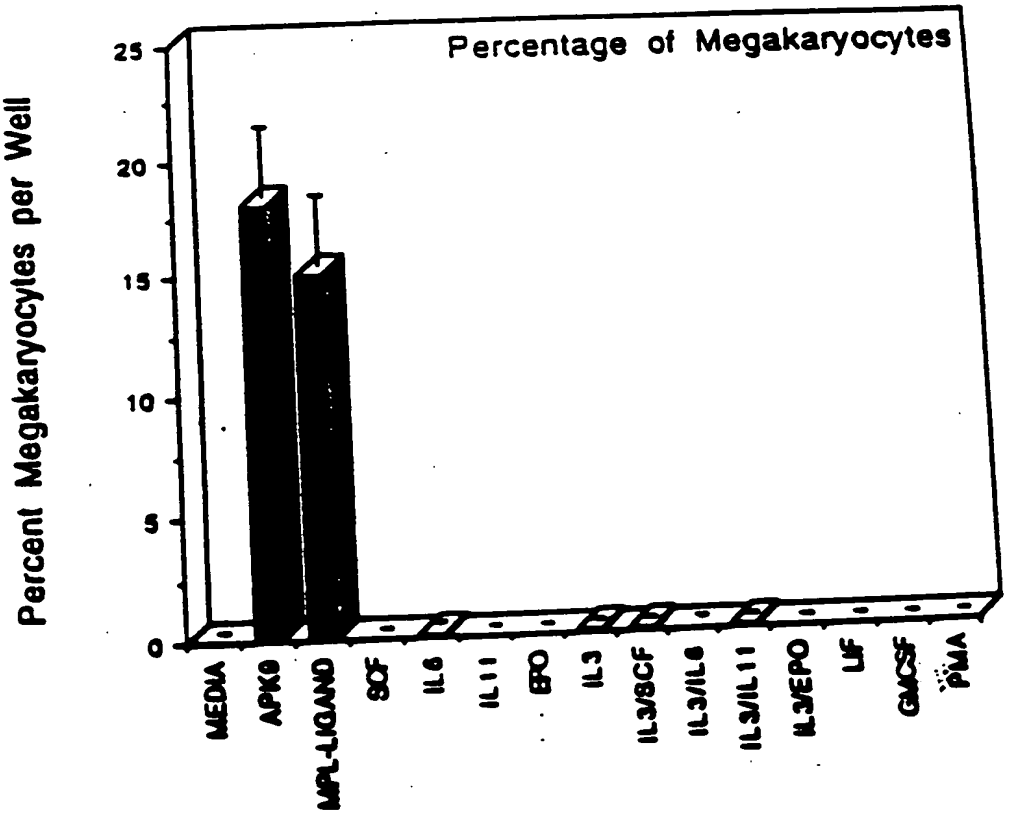

Percentage of Megakaryocytes
Percent Megakaryocytes per Well
25
20
15
10
5
0
MEDIA
APK6
MPL-LIGAND
SCF
IL6
IL11
EPO
IL3
IL3/SCF
IL3/IL6
IL3/IL11
IL3/EPO
LIF
GMCSF
PMA

Figure 9

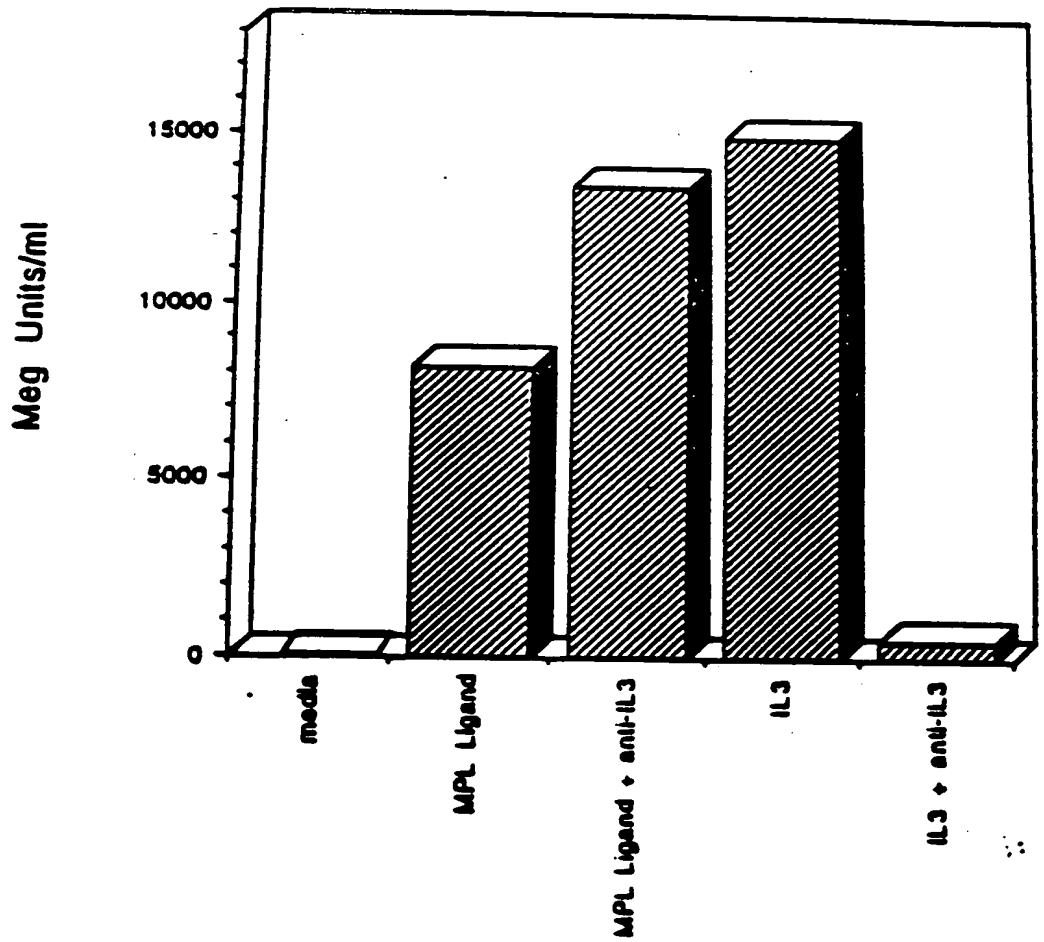

Meg Units/ml
15000
10000
5000
0
media
MPL Ligand
MPL Ligand + anti-IL3
IL3
IL3 + anti-IL3

Figure 10

MDGF-1

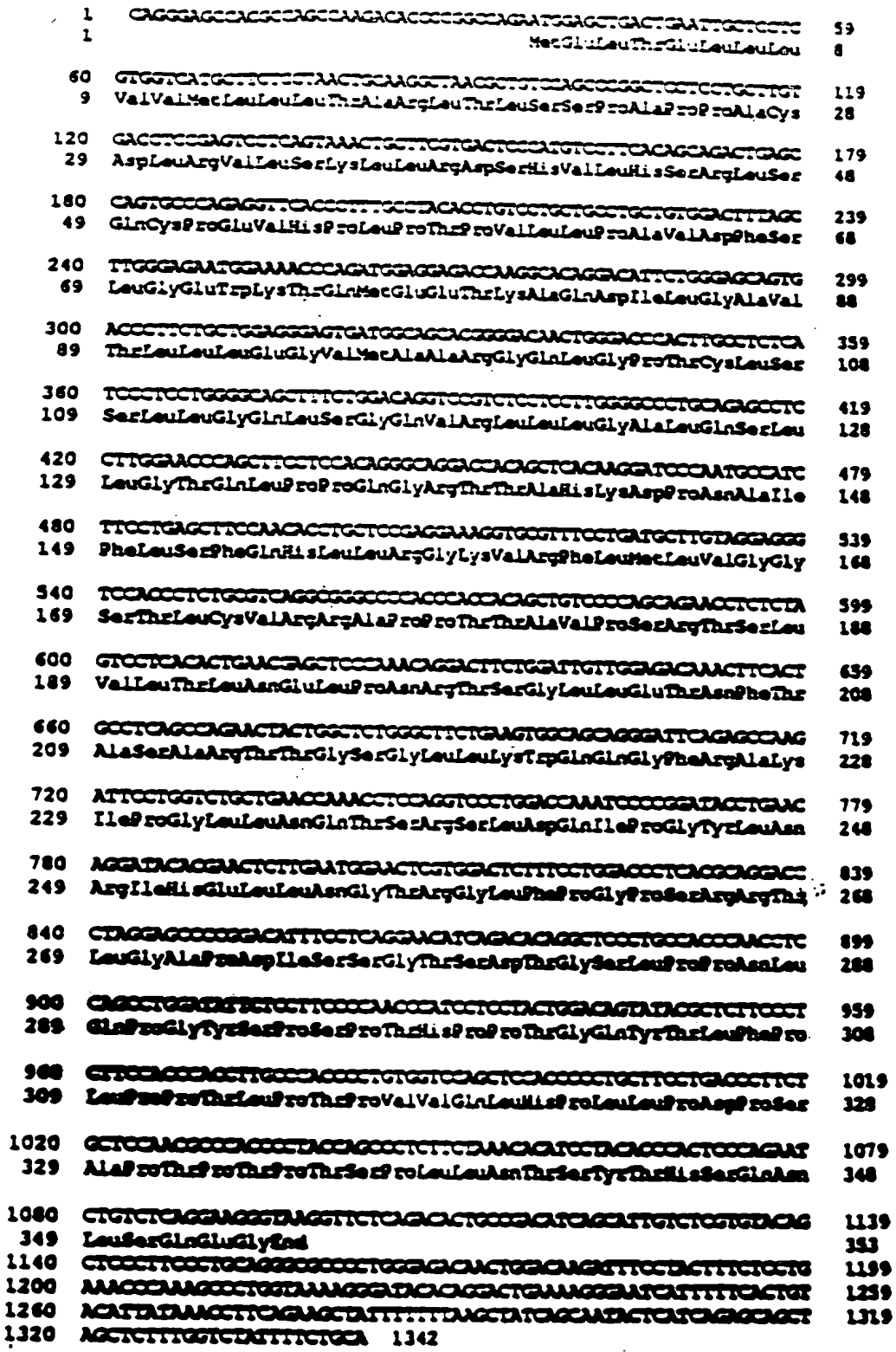


Figure 11

Human MDGF cDNA (no IVS 5)

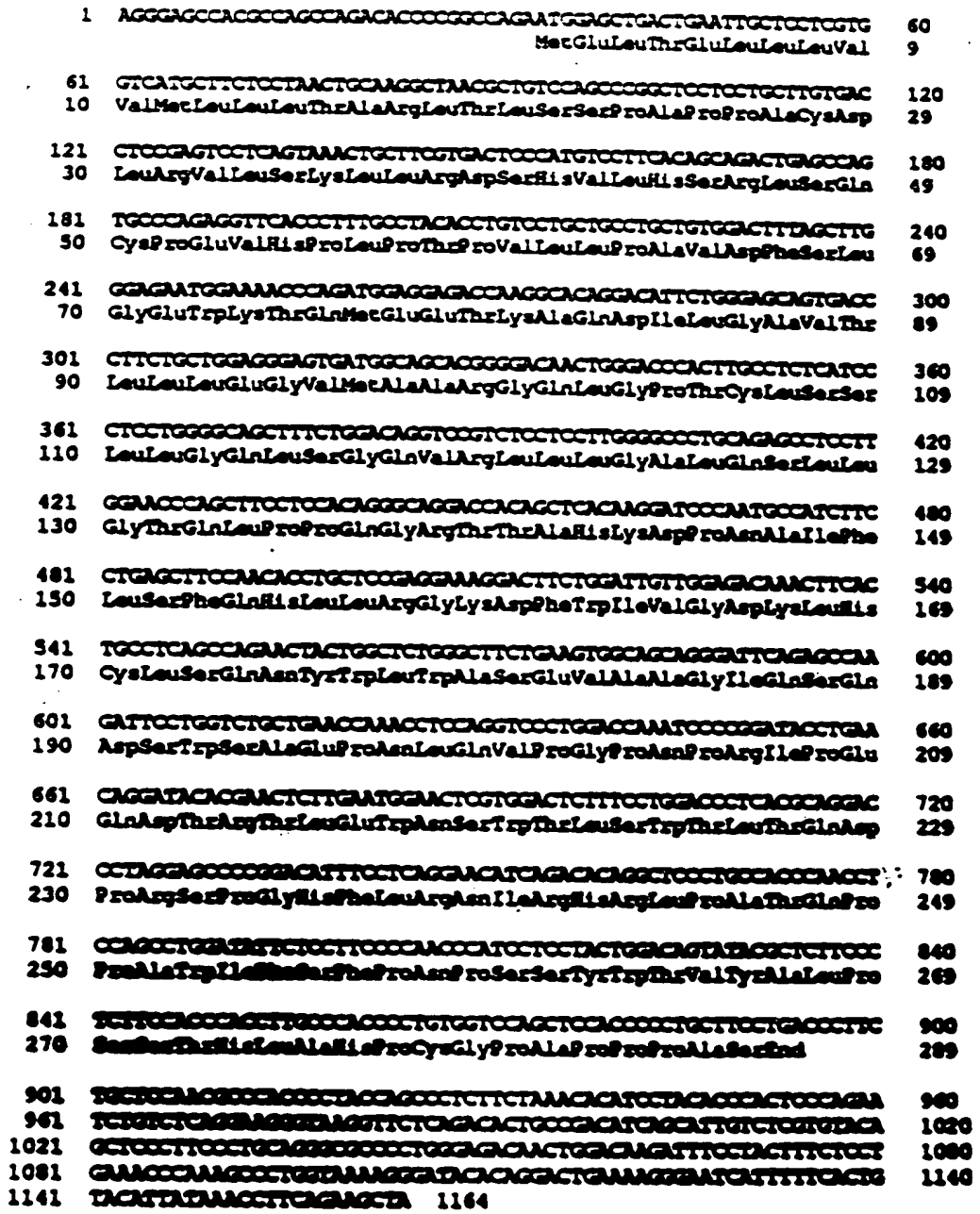


Figure 12

Percent Similarity: 83.003 Percent Identity: 76.487

Dogmdgf.Pep x Mdgf.Pep May 24, 1994 17:13 ..

```
canine   1 MELTELLLVVMLLLTARLDPCLPAPPACDPRLLNKMLRDSHVLHSRLSQC  50
           |||||||||||||||||||. : ||||||| |:|.|:|||||||||||||||
human    1 MELTELLLVVMLLLTARLTLSSPAPPACDLRVLSKLLRDSHVLHSRLSQC  50

          51 PDIYPLSTPVLLPAVDFSLGEWKTQKEQTKAQDVWGAVALLLDGVLAARG 100
             |::.||.|||||||||||||||||.|:||||::|||.|||:||:||||
          51 PEVHPLPTPVLLPAVDFSLGEWKTQMEETKAQDILGAVTLLLEGVMAARG 100

         101 QLGPSCLSSLLGQLSGQVRLLLGALQGLLGTQLPPQGRTTTHKDPNAIFL 150
             ||||.|||||||||||||||||||:|||||||||||||.|||||||||
         101 QLGPTCLSSLLGQLSGQVRLLLGALQSLLGTQLPPQGRTTAHKDPNAIFL 150

         151 SFQQLLRGKVRFLLLVAGPTLCAKQSQPTTAVPTNTSLFLTLRKLPNRTS 200
             |||:||||||||||:||:|.|||.:...||||||..|||.|||..||||||
         151 SFQHLLRGKVRFLMLVGGSTLCVRRAPPTTAVPSRTSLVLTLNELPNRTS 200

         201 GLLETNSSISARTTGSGLLKRLQGFRAKIPGLLNQTSRSLNQTPGHLSRT 250
             |||||| . |||||||||||: ||||||||||||||||||:|.||.|.|.
         201 GLLETNFTASARTTGSGLLKWQQGFRAKIPGLLNQTSRSLDQIPGYLNRI 250

         251 HGPLNGTHGLLPGLSLTALGAPDIPPGTSDMDALPPNLWPRYSPSPIHPP 300
             |: ||||:||:|| |  .|||||..|||| :.||||| | |||||.|||
         251 HELLNGTRGLFPGPSRRTLGAPDISSGTSDTGSLPPNLQPGYSPSPTHPP 300

         301 PGQYTLFSPLPTSPTPQNPLQPPPPDPSA.TANSTSPLLIAAHPHFQNLS 349
             .|||||||.  || |||  .|:|  ||||| |:..||||| ....| ||||
         301 TGQYTLFPLPPTLPTPVVQLHPLLPDPSAPTPTPTSPLLNTSYTHSQNLS 350

         350 QEE 352
             |||
         351 QEG 353
```

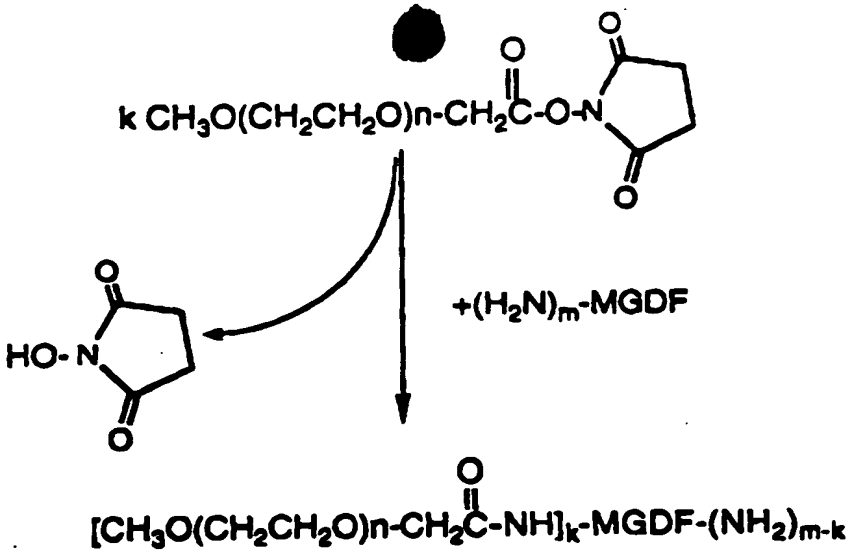
Figure 13A

Percent Similarity: 79.603 Percent Identity: 72.521

Mousemdgf.Pep x Mdgf.Pep May 23, 1994 12:27 ..

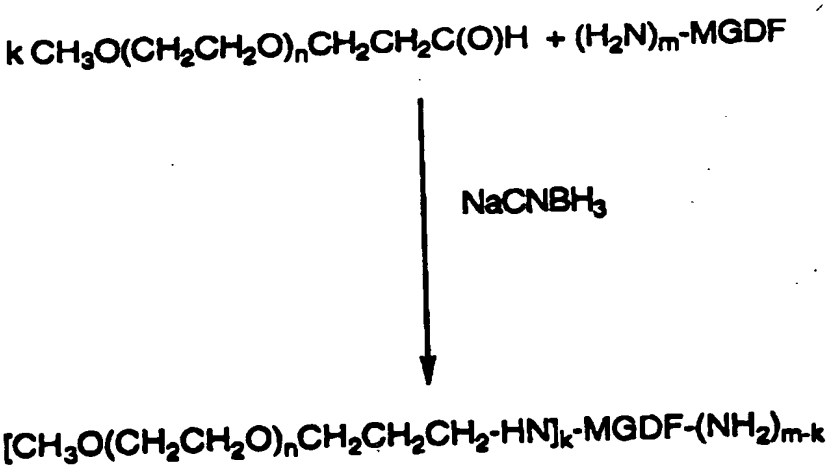
```
murine   1 MELTDLLLAAMLLAVARLTLSSPVAPACDPRLLNKLLRDSHLLHSRLSQC  50
           ||||:|||..||| .||||||||.:|||| |:|.|||||||:||||||||
human    1 MELTELLLVVMLLLTARLTLSSPAPPACDLRVLSKLLRDSHVLHSRLSQC  50

          51 PDVDPLSIPVLLPAVDFSLGEWKTQTEQSKAQDILGAVSLLLEGVMAARG 100
             |:|.||..|||||||||||||||||| |:.|||||||||.||||||||||
          51 PEVHPLPTPVLLPAVDFSLGEWKTQMEETKAQDILGAVTLLLEGVMAARG 100

         101 QLEPSCLSSLLGQLSGQVRLLLGALQGLLGTQLPLQGRTTAHKDPNALFL 150
             ||:|.|||||||||||||||||||:||||||| ||||||||||||:||
         101 QLGPTCLSSLLGQLSGQVRLLLGALQSLLGTQLPPQGRTTAHKDPNAIFL 150

         151 SLQQLLRGKVRFLLLVEGPTLCVRRTLPTTAVPSSTSQLLTLNKFPNRTS 200
             |:|:||||||||||:||:|.|||||||. |||||||.|| :|||||.:|||||
         151 SFQHLLRGKVRFLMLVGGSTLCVRRAPPTTAVPSRTSLVLTLMELPNRTS 200

         201 GLLETNFSVTARTAGPGLLSRLQGFRVKITPGQLNQTSRSPVQISGYLNR 250
             |||||||...|||.|.|||.: ||||.|| || ||||||| ||.||||||
         201 GLLETNFTASARTTGSGLLKWQQGFRAKI.PGLLNQTSRSLDQIPGYLNR 249

         251 THGPVNGTHGLFAGTSLQTLEASDISPGAFNKGSLAFNLQGGLPPSPSLA 300
             .|: :|||:|||:|.| .||:|.|||.|. :.|||: |||.|..|||. :
         250 IHELLNGTRGLFPGPSRRTLGAPDISSGTSDTGSLPPNLQPGYISPSPTHP 299

         301 PDGH.TPFPPSPALPTTHGSPPQLHPLFPDPSTTMPMSTAPHPVTHXPHP 349
             |.|: | || .|.||| ...|||||:||||.. |..|.| | |.|.
         300 PTGQYTLFPLPPTLPT...PVVQLHPLLPDPSAPTPTPTSPLLNTSYTHS 346

         350 RNLSQET 356
             .||||||.
         347 QNLSQEG 353
```

Figure 13B



k, m and n are the same as defined in Figure 15.

Figure 14



k - number of PEG molecules reacted with a molecule of MGDF;

n - degree of polymerization of PEG used in the reaction; e.g. n=2000 for PEG of MW=100 kD; n=40 for PEG of MW=2 kD.

m - total number of primary amino groups per MGDF molecule.

Figure 15

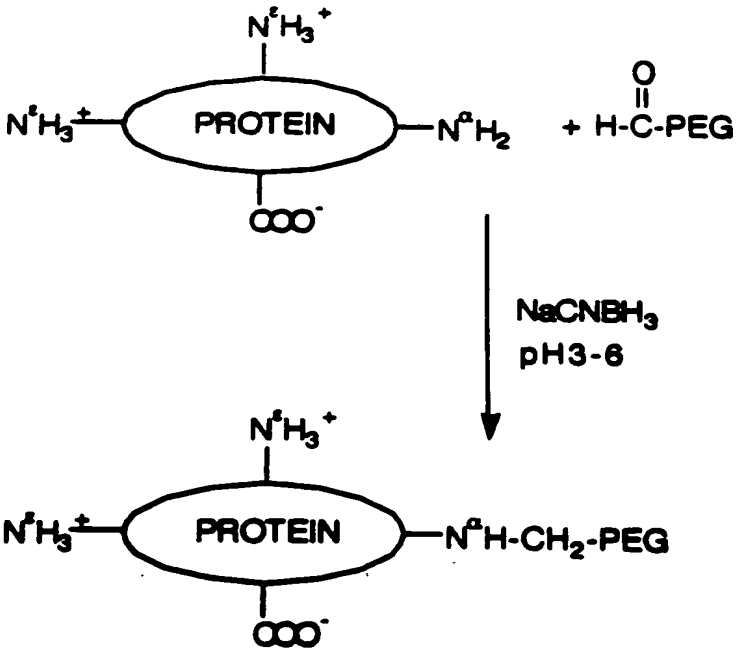

$N^{\varepsilon}H_3^+$
$N^{\varepsilon}H_3^+$
PROTEIN
$N^{\alpha}H_2$
+ H-C-PEG
O
COO^-
$NaCNBH_3$
pH3-6
$N^{\varepsilon}H_3^+$
$N^{\varepsilon}H_3^+$
PROTEIN
$N^{\alpha}H$-CH_2-PEG
COO^-

Figure 16

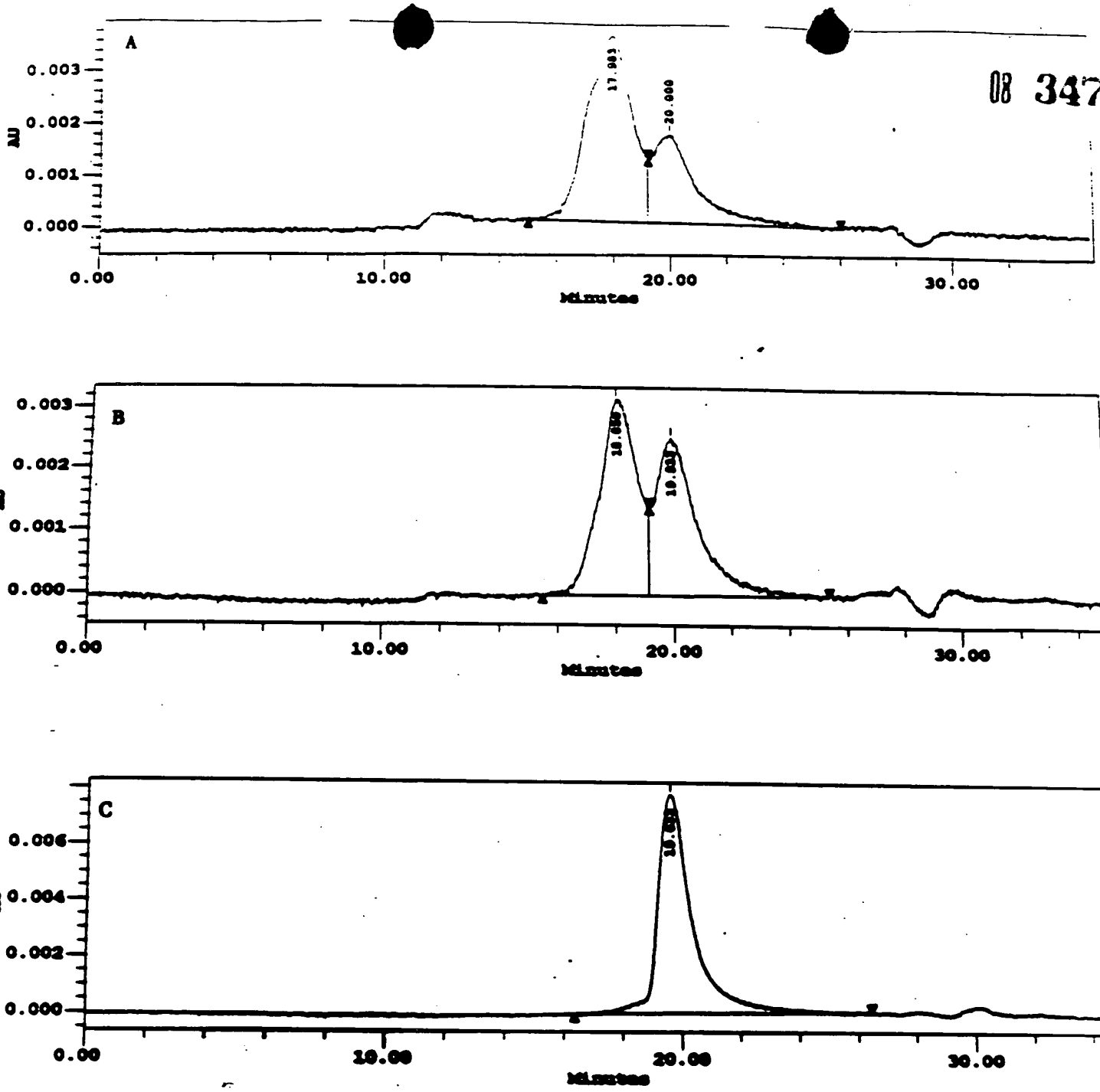

A
0.003
0.002
0.001
0.000
AU
17.983
20.000
0.00
10.00
20.00
30.00
Minutes
B
0.003
0.002
0.001
0.000
AU
18.650
19.833
0.00
10.00
20.00
30.00
Minutes
C
0.006
0.004
0.002
0.000
AU
18.650
0.00
10.00
20.00
30.00
Minutes

Figure 17

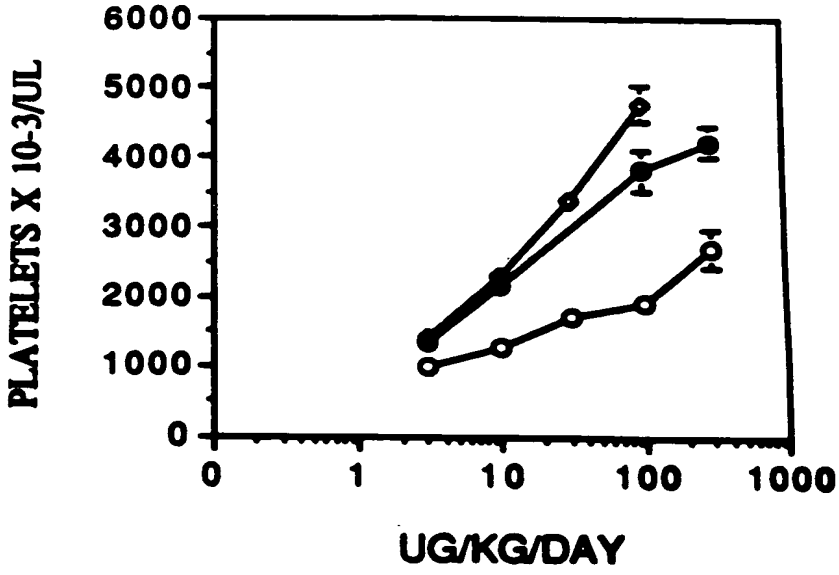

6000
5000
4000
3000
2000
1000
0
PLATELETS X 10-3/UL
0
1
10
100
1000
UG/KG/DAY

Figure 18

Purification Flow Chart for r-HuMGDF

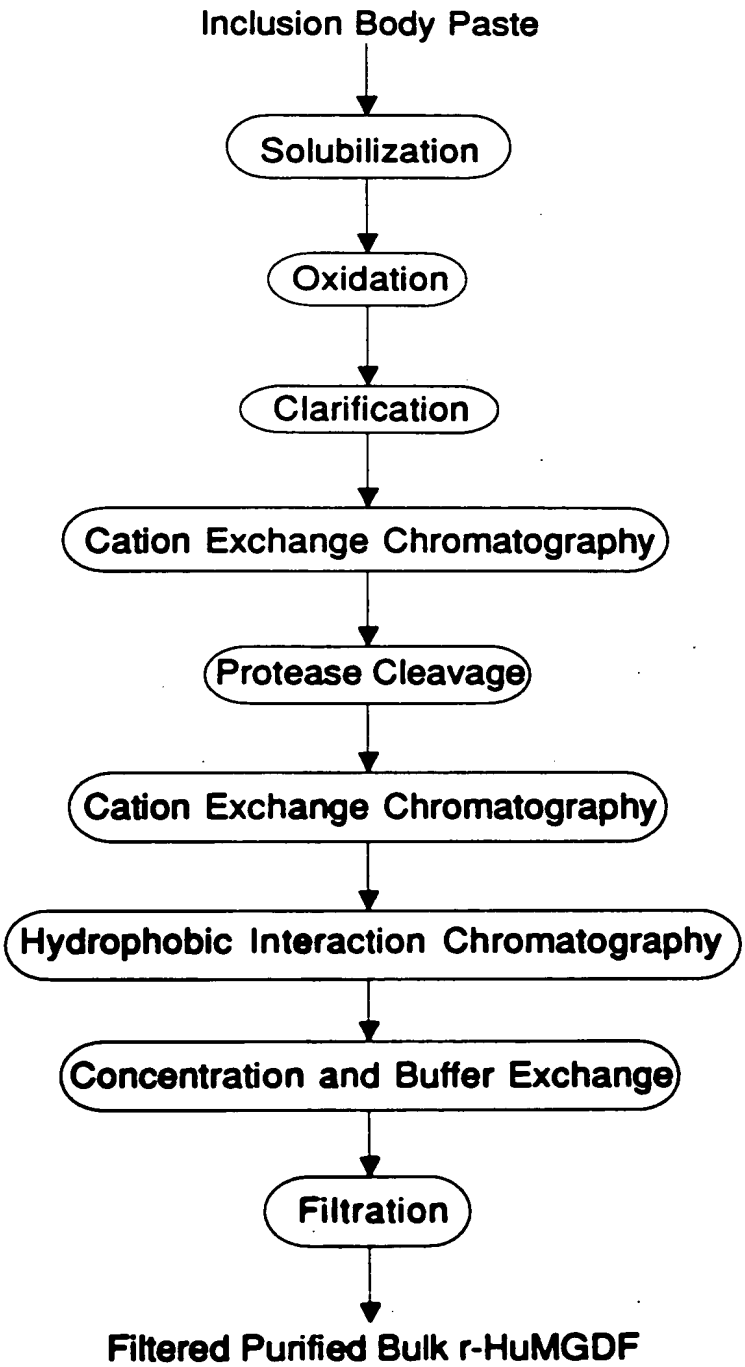


Figure 19

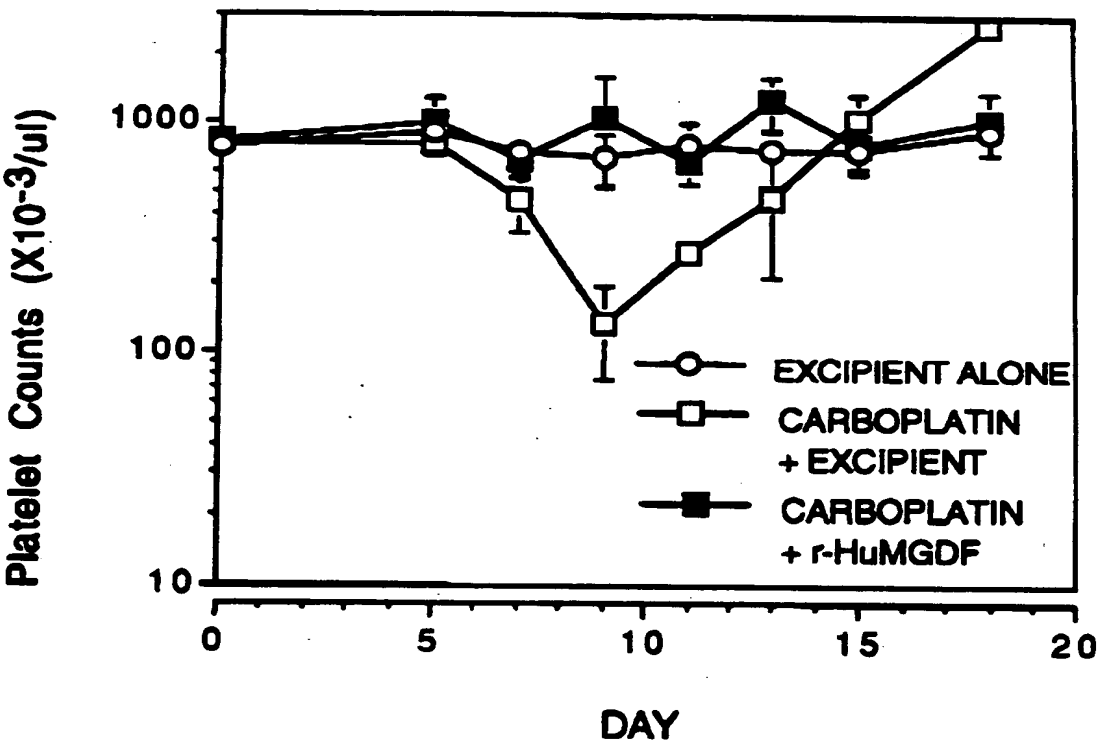

Platelet Counts (X10-3/ul)
1000
100
10
0
5
10
15
20
DAY
EXCIPIENT ALONE
CARBOPLATIN
+ EXCIPIENT
CARBOPLATIN
+ r-HuMGDF

Figure 20

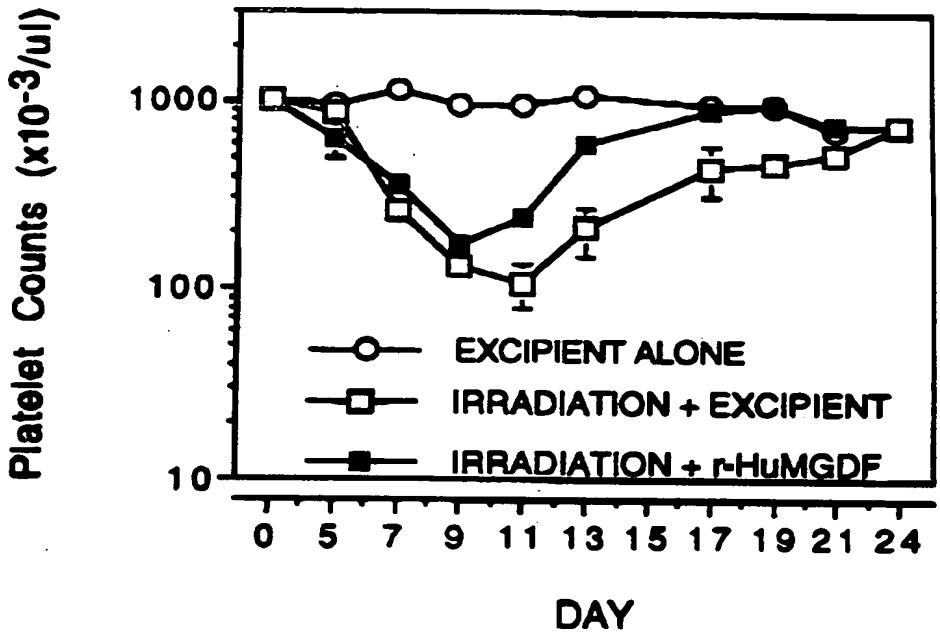

Platelet Counts (x10-3/ul)
1000
100
10
EXCIPIENT ALONE
IRRADIATION + EXCIPIENT
IRRADIATION + r-HuMGDF
0 5 7 9 11 13 15 17 19 21 24
DAY

Figure 21

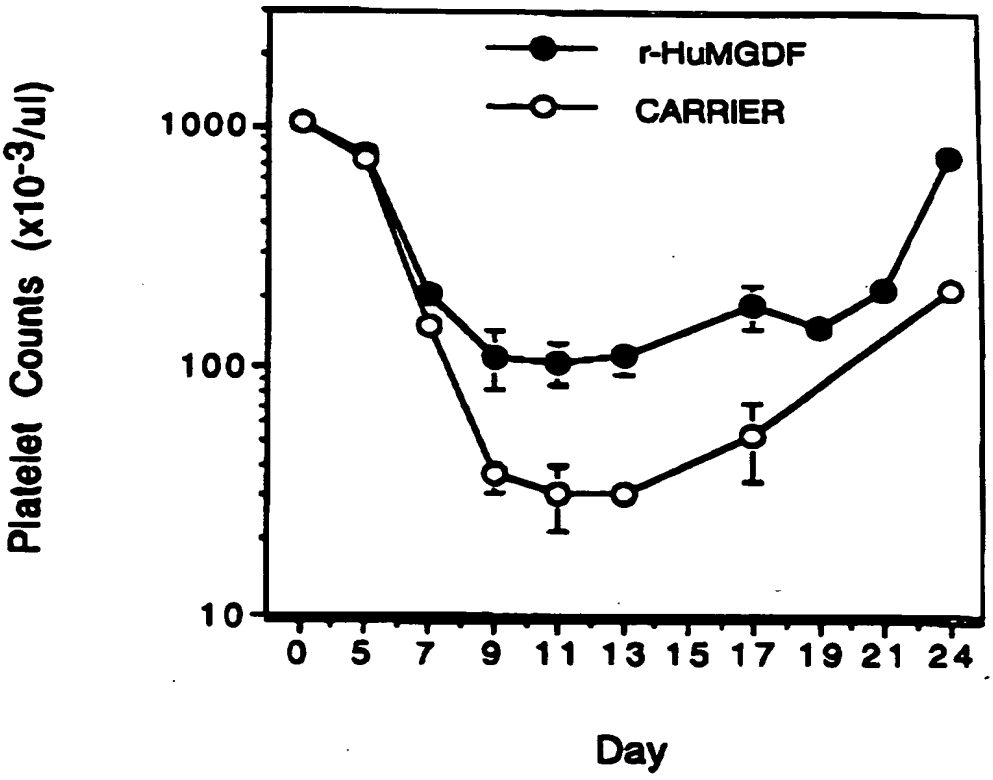

r-HuMGDF
CARRIER
Platelet Counts (x10-3/ul)
1000
100
10
0 5 7 9 11 13 15 17 19 21 24
Day

Figure 22

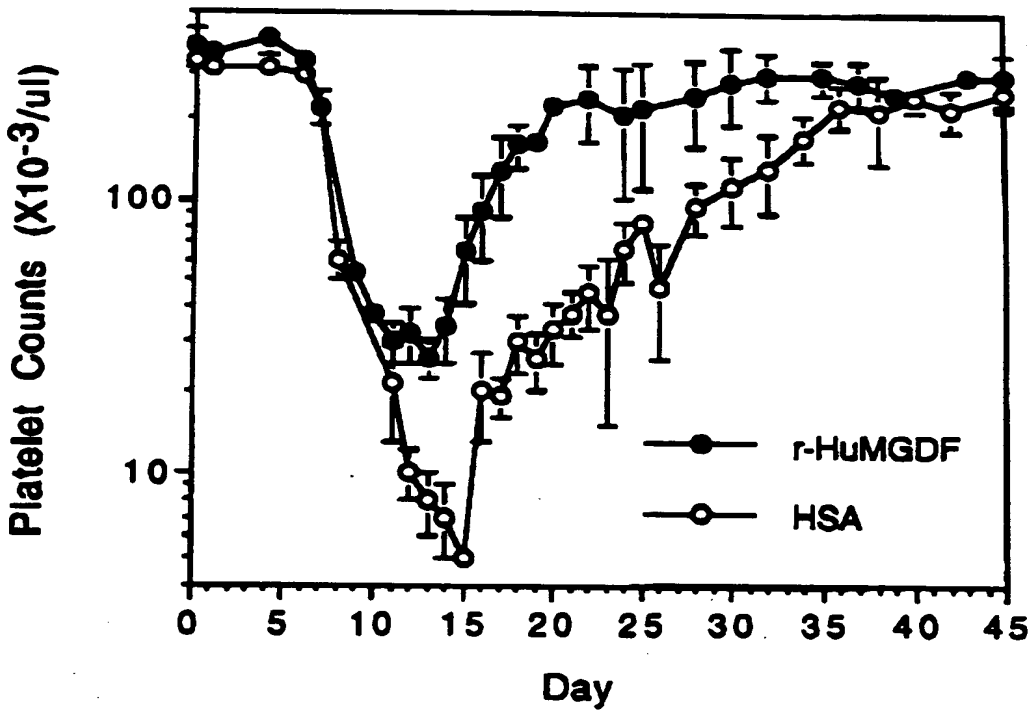

Platelet Counts (X10-3/ul)
100
10
0
5
10
15
20
25
30
35
40
45
Day
r-HuMGDF
HSA

Figure 23

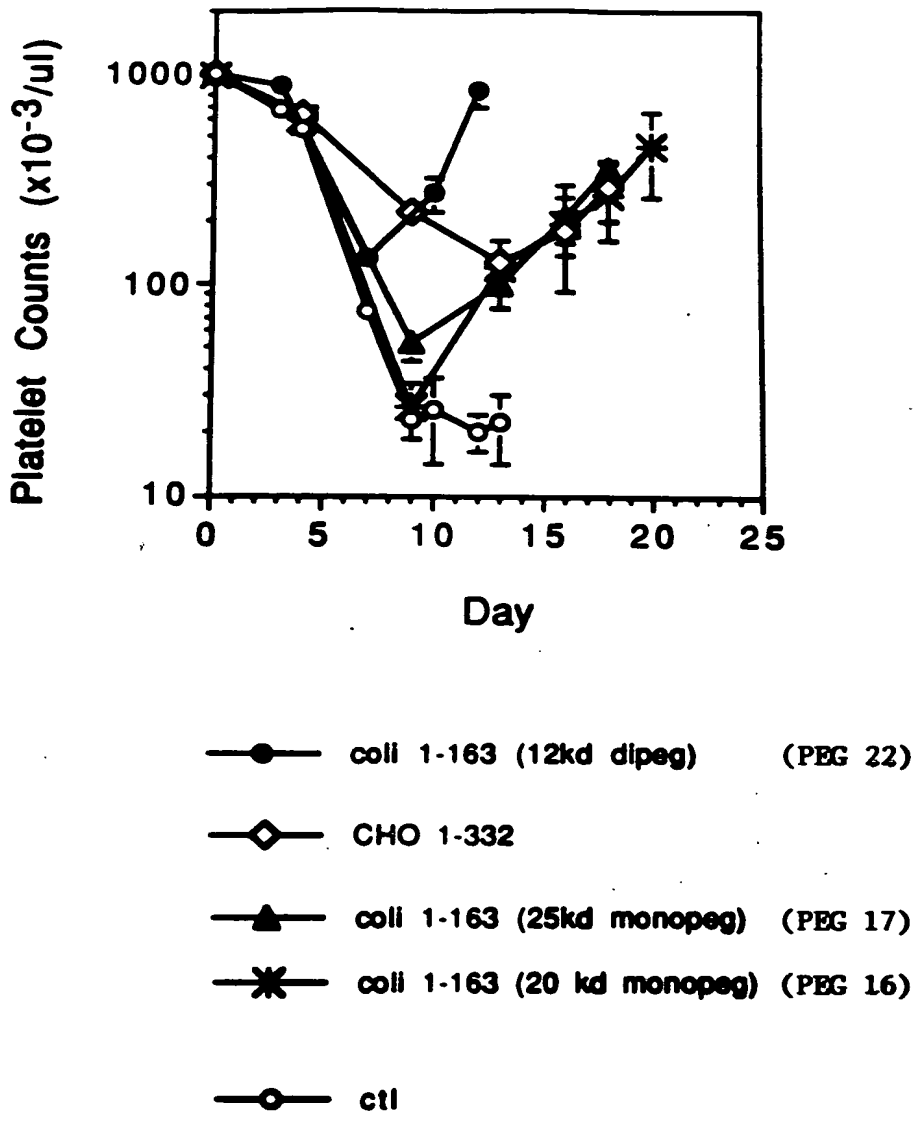

Platelet Counts (x10-3/ul)
1000
100
10
0
5
10
15
20
25
Day
coli 1-163 (12kd dipeg) (PEG 22)
CHO 1-332
coli 1-163 (25kd monopeg) (PEG 17)
coli 1-163 (20 kd monopeg) (PEG 16)
ctl

Figure 24

r-HuMGDF (1-163) Translation

```
ATG AAA AGT CCT GCA CCA CCT GCA TGT GAT TTA CGG GTC CTG
MET LYS SER PRO ALA PRO PRO ALA CYS ASP LEU ARG VAL LEU

TCT AAA CTG CTG CGC GAC TCT CAC GTG CTG CAC TCT CGT CTG
SER LYS LEU LEU ARG ASP SER HIS VAL LEU HIS SER ARG LEU

TCC CAG TGC CCG GAA GTT CAC CCG CTG CCG ACC CCG GTT CTG
SER GLN CYS PRO GLU VAL HIS PRO LEU PRO THR PRO VAL LEU

CTT CCG GCT GTC GAC TTC TCC CTG GGT GAA TGG AAA ACC CAG
LEU PRO ALA VAL ASP PHE SER LEU GLY GLU TRP LYS THR GLN

ATG GAA GAG ACC AAA GCT CAG GAC ATC CTG GGT GCA GTA ACT
MET ALA ALA ARG LYS ALA GLN ASP ILE LEU GLY ALA VAL THR

CTG CTT CTG GAA GGC GTT ATG GCT GCA CGT GGC CAG CTT GGC
LEU LEU LEU GLU GLY VAL MET ALA ALA ARG GLY GLN LEU GLY

CCG ACC TGC CTG TCT TCC CTG CTT GGC CAG CTG TCT GGC CAG
PRO THR CYS LEU SER SER LEU LEU GLY GLN LEU SER GLY GLN

GTT CGT CTG CTG CTC GGC GCT CTG CAG TCT CTG CTT GGC ACC
VAL ARG LEU LEU LEU GLY ALA LEU GLN SER LEU LEU GLY THR

CAG CTG CCG CCA CAG GGC CGT ACC ACT GCT CAC AAG GAT CCG
GLN LEU PRO PRO GLN GLY ARG THR THR ALA HIS LYS ASP PRO

AAC GCT ATC TTC CTG TCT TTC CAG CAC CTG CTG CGT GGC AAA
ASN ALA ILE PHE LEU SER PHE GLN HIS LEU LEU ARG GLY LYS

GTT CGT TTC CTG ATG CTG GTT GGC GGT TCT ACC CTG TGC GTT
VAL ARG PHE LEU MET LEU VAL GLY GLY SER THR LEU CYS VAL

CGT CGG GCG CCG CCA ACC ACT GCT GTT CCG TCT TAA
ARG ARG ALA PRO PRO THR THR ALA VAL PRO SER STOP
```

Figure 25